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Separation and preconcentration of trace level of lead in one drop of blood sample by using graphite furnace atomic absorption spectrometry

Kamlesh Shrivas [a,*], Devesh Kumar Patel [b]

[a] Center for Biologics Evaluation and Research, Food and Drug Administration, 8800 Rockville pike, Building 29A, 2B20, Bethesda, MD 20892, United States
[b] Department of chemistry, Govt. Science College, Rajnandgaon-491441, CG, India

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ABSTRACT

Drop-to-drop solvent microextraction (DDSME) assisted with ultrasonication is applied for the determination of lead in one drop (30 μL) of blood sample by using graphite furnace atomic absorption spectrometry (GF-AAS). The optimum extraction efficiency of lead was observed for 10 min extraction time at pH 5.0 with 2 μL of organic solvent that containing 0.5 M of Cyanex-302. The optimized methodology exhibited good linearity in the range of 0.3–30.0 ng mL^{-1} lead with relative standard deviations (RSD) from 2.5 to 4.4%. The method is found to be simple and rapid for the analysis of lead in micro amount of blood sample with the limit of detection (LOD) of 0.08 ng mL^{-1}. The application of the proposed method has been successfully tested for the determination of lead in blood samples. The results showed that under the optimized experimental conditions, the method showed good sensitivity and recovery %, as well as advantages such as linearity, simplicity, low cost and high feasibility.

1. Introduction

Lead and inorganic lead compounds are found in a variety of commercial products and industrial materials, including paints, plastics, storage batteries, bearing alloys, insecticides, and ceramics [1]. However, nowadays the most important source of lead in our environment is anthropogenic activities such as burning fossil fuels, mining and various manufacturing processes. Lead poisoning is a medical condition that caused by increased levels of the lead in the blood. Lead may causes irreversible neurological damage as well as renal disease, cardiovascular effect, and reproductive toxicity. Moreover, exposure to high level of lead can severely damage the kidney and brain. The International Agency for Research on Cancer (IARC) has determined that inorganic lead is probably carcinogenic to human. Thus, the determination of lead in environmental and biological samples at ultra trace level is very important [2–4].

There are several methods reported for the determination of lead in various samples, including electro thermal atomic absorption spectrometry (ET-AAS) [5], inductively coupled plasma atomic emission spectrometry (ICP-AES) [6], inductively coupled plasma mass spectrometry (ICP-MS) [7], and spectrophotometry [8]. However, the determination of lead at very low concentrations is often difficult because of insufficient sensitivity of method as well as the matrix interferences occurring during the real sample analysis. For this reason, a preliminary separation and preconcentration step are often required to enhance the sensitivity of the method. Liquid–liquid extraction (LLE), solid phase extraction (SPE), and cloud point extraction (CPE) are traditional methods for separation and preconcentration of lead [9]. Soylak et al. demonstrated the use of Diaion HP-2MG (methacrylic ester copolymer) as a solid phase extracting probe for separation and preconcentration of metals ions from different types of samples [10,11].

In order to reduce the consumption and exposure to hazardous organic solvents, disposal costs and extraction time, liquid-phase microextraction (LPME) such as single-drop microextraction (SDME) [12,13], hollow fiber liquid-phase microextraction (HF-LPME) [14–16], dispersive liquid-liquid microextraction (DLLME), and drop-to-drop solvent microextraction (DDSME) [17–19] are alternative for the classical solvent–solvent extraction methods. The most recent trends include LPME, a miniaturization of the LLE. This novel technique is based on the distribution of analytes between the micro amount of organic solvent and aqueous sample solution. The organic solvent is first exposed to the sample solution and target analytes are extracted from the sample matrix into the organic acceptor phase. After the equilibrium is reached, the solvent with concentrated analytes is then transferred to the instrument for further analysis [13,18,19]. These sample procedures generally eliminate the disadvantages of conventional extraction methods, such as time consuming operation and using of specialized apparatus. But, the amount of sample solution which is used in these techniques is large (≈1–20 mL), which is not suitable for the analysis of biological samples like, blood, plasma, etc., where the amount of sample is very minute. Thus, another approach for the sample preparation is needed in order to reduce the sample size.

* Corresponding author.
E-mail address: shrikam@rediffmail.com (K. Shrivas).

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DDSME is simple and rapid sample preparation technique where the micro amount (15–30 μL) of biological sample is used for the analysis of target compound [17,19]. In the present work, 30 μL of blood sample is used to extract lead in to 2 μL of toluene containing Cyanex-302 and analyzed by using GF-AAS.

2. Experimental

2.1. Reagents and materials

The entire reagents used were of analytical reagent grade. Toluene and cyclohexane, were purchased from Mallinckrodt (Paris, KY, USA). Sodium chloride, chloroform, iso-octane iso-obutane and n-hexane were purchased from Merck (Darmstadt, Germany). Cyanex-302 was supplied by Cytec Industries Inc. (Markham, ON, Canada). The different pH buffer solution (sodium citrate of 2.0, 4.0 and 5.0, sodium phosphate of 7.0 and 3-(cyclohexylamino)-1-propane sulfonic acid of 9.0 were obtained from Fluka (Buchus, Switzerland). Certified reference material (CRM), Lot No. 194 and 195 of bovine blood was obtained from the Community Bureau of Standards (BCR), B-1049 Brussels. Standard stock solution of lead (II) was prepared by dissolving the appropriate amount of lead nitrate in distilled water containing 0.5 mL of concentrated HNO_3. The solution was standardized by EDTA complexometric method and the working solutions were prepared by the dilution of stock solution. A 1.0 M stock solution of Cyanex-302 [bis(2,4,4-trimethylpentyl monothiophosphinic acid)] was prepared by dissolving the substance in organic solvent toluene.

2.2. Graphite furnace-atomic absorption spectrometry

Shimadzu model AA6300G atomic absorption spectrometer (Kyoto, Japan) with graphite furnace (GFA-EX7i), a lead hallow cathode lamp as radiation source (Hamamatsu Photonics, Japan) at the 283.3 nm wavelength with a slit width of 0.4 nm, 2 mA current and deuterium background corrector was used for measurement. The temperature program used to determine the lead by GF-AAS is shown in Table 1. Argon was used as the inert gas at 300 mL min^{-1} (drying and ashing) except during the atomization step; the flow was stopped and 2000 mL min^{-1} while cleaning condition.

2.3. Sample collection and preparation for analysis of lead in blood

Blood samples were collected in cleaned polyethylene bottle from five different persons and stored in refrigerator until the analysis of samples. A 1 mL of the blood sample was treated with sulfuric acid and nitric acid, and then heated nearly to dryness. The residue obtained was diluted in appropriate amount of distilled water according to concentration of lead present in the sample solution and then filtered with Whatman filter paper. A 30 μL sample solution was used for the determination of lead by using DDSME/GF-AAS.

Table 1
Graphite furnace temperature program for the determination of lead.

Stage	Furnace temperature (°C)	Ramp times (s^{-1})	Hold time (s^{-1})
Drying	120	5	10
Ashing	300	5	4
Atomization	1700	0	4
Cleaning	2500	0	2

2.4. Procedure for the DDSME extraction of lead

The general DDSME procedure for the extraction of lead was as follows: 30 μL of the pretreated blood sample or standard solution placed in a 100 μL glass vial which was tightly sealed with a silicone septum. The pH of the sample solution was maintained by using either 1 μL buffer solution of 2.0 (sodium citrate), 4.0 (sodium citrate), 5.0 (sodium citrate), 7.0 (sodium phosphate) and 9.0 (3-(cyclohexylamino)-1-propane sulfonic acid) as described in the text. Then, the vial with sample solution was put on ultrasonic bath for the agitation of sample at room temperature. Microsyringe filled with 2 μL of toluene that containing a Cyanex-302 was inserted in the vial through the septum until its needle tip was put into the sample solution. The plunger was depressed and microdrop of the acceptor phase was exposed for 10 min. After the extraction, the drop was retracted and subsequently injected into the graphite furnace for subsequent determination of lead.

3. Results and discussion

3.1. Effect of organic solvent and volume of acceptor phase

The choice of the organic solvent should be based on the several considerations. First, it should have a low solubility in water so as to prevent its dissolution into the aqueous phase, and a low volatility, which will restrict the solvent evaporation during the extraction. Second, it should provide high extraction recoveries of target analytes. On the basis of these considerations, n-hexane, cyclohexane, iso-octane, chloroform and toluene were tested in the present work to extract the lead from the sample solution. Toluene was found to be better for the extraction of lead from sample solution and used for further experiments. The reason may be due to the higher solubility of lead-Cyanex complex in to the toluene and also this solvent could be remain longer time at the tip of syringe than any other solvents tested in the experiments. The effect of volume of acceptor phase (organic solvent) on the extraction efficiency was also examined in the range of 0.5–2.0 μL for 10 min of extraction time. Extraction efficiency of lead was increased as the exposure volume of extraction phase increased to 2.0 μL. However, there was chance of miscibility of aqueous phase as the volume of exposure acceptor phase increased more than 2 μL which caused the poor precision (RSD, >±15%) of the results. Hence, 2.0 μL of volume of organic solvent was selected for further experiments.

3.2. Extraction time

The extraction of analytes at a given time is dependent on the mass transfer of analyte from aqueous samples to the organic solvent drop. This procedure requires a period of time for equilibrium. The impact of extraction time, 2–15 min was investigated for the extraction of lead from spiked sample solution with agitation by using ultrasonic bath. As can be seen in Fig. 1, the relative peak area of lead was increased with extraction time up to 10 min. After 10 min, the decrease in the extraction efficiency was observed due to the dissolution of solvent into the aqueous phase. Therefore, 10 min extraction time was used for all subsequent experiments.

3.3. Influence of the sample pH

Adjustment of the pH can enhance the extraction of the target analytes from sample solution into the organic solvent containing Cyanex-302 reagent. Thus, 1 μL of different buffer solution (2.0, 4.0, 5.0, 7.0 and 9.0) was added to sample solution in order to see the effect of pH on the extraction of lead. Thus, the extraction efficiency of lead was studied as a function of pH. Extraction of lead

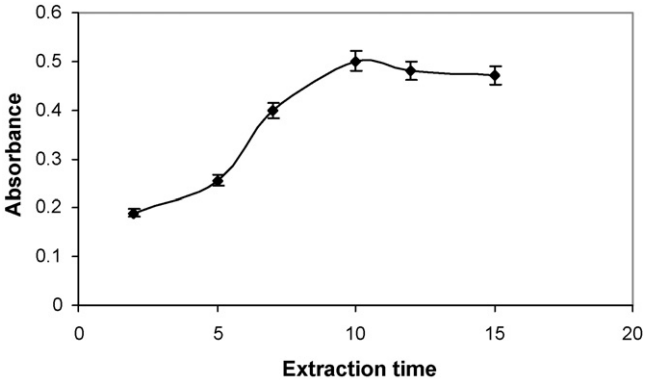

Fig. 1. Effect of extraction time on the extraction of lead by using 2 μL of toluene containing a 0.5 M Cyanex-302 at pH 5.0 under the ultrasonication.

into the organic solvent containing Cyanex-302 was largely governed by the change in the pH of the aqueous solution. A series of experiments were performed to optimize the pH of sample solution from 2.0 to 10.0 at room temperature for 10 min extraction time. As shown in Fig. 2, the extraction efficiency of analyte was increased with an increase in the pH from 2.0 to 5.0. When the pH of sample was below or above 5.0, the extraction efficiency began to decrease and this might be due to the incomplete extraction of lead–Cyanex complex. However, the reason for the higher extraction of lead at pH 5.0 may be due to the higher stability of lead–Cyanex complex at this pH value. Thus, pH 5.0 was selected for further investigations.

3.4. Effect of Cyanex-302 reagent concentration

The concentration of Cyanex-302 influences the extraction efficiency of target analytes from the sample solution. Therefore, the dependence of analytical signal intensity of the target analytes on the Cyanex-302 concentration was studied. It was found that the analytical signal intensity was increased with the increase of Cyanex-302 concentration from 0.05 to 0.5 M and there after no change in the signal intensity was observed, shown in Fig. 3. Thus, 0.5 M of Cyanex-302 was sufficient for separation and preconcentration of lead from the sample solution.

3.5. Linear range, limit of detection, preconcentration factor, precision of the method

Linearity, limit of detection (LOD), correlation coefficient (R^2), preconcentration factor (EF), and precision of the proposed method

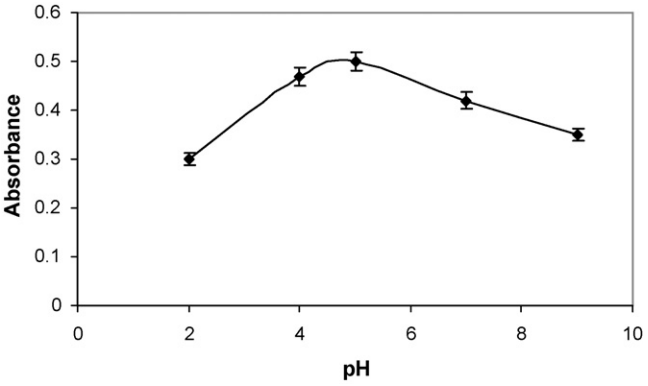

Fig. 2. Effect of pH on the extraction of lead for 10 min extraction time and using 2 μL of toluene containing a 0.5 M Cyanex-302 under the ultrasonication.

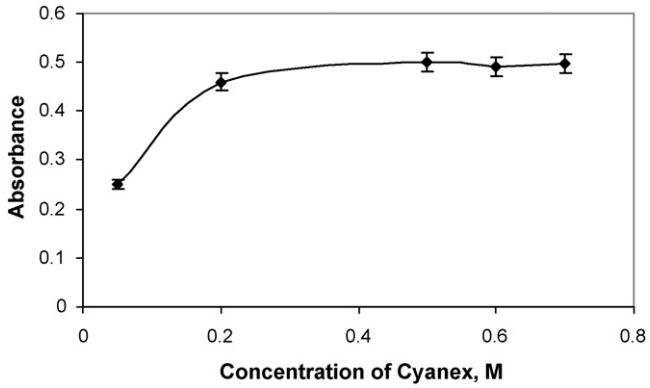

Fig. 3. Effect of Cyanex-302 concentration on the extraction of lead for 10 min extraction time and using 2 μL of toluene containing a Cyanex-302 at pH 5.0 under the ultrasonication.

were investigated at optimized conditions. Linearity was observed over the range of 0.5–30.0 ng mL^{-1} with a correlation coefficient (R^2) of 0.996. The slope and intercept value of the calibration were 0.0317 and 0.0072, respectively. The LOD, based on a signal-to-noise ratio (S/N) of 3, was 0.08 ng mL^{-1}. The EF was calculated as the ratio of final concentration of the analyte in the organic solvent to the concentration of analyte in the original solution. For, these three replicate extractions were performed at optimal conditions in blood sample containing 5 ng mL^{-1} of lead. The PF for lead in blood sample was found to be 13. The precision (relative standard deviation, RDS) of the method was assessed by carrying out a six replicate analysis of standard solution of 5 ng mL^{-1} of lead and the RSD of the method was found to be 3.4%.

3.6. Real samples analysis

The present method was successfully applied for the determination of lead in blood samples that were collected from five different persons. A 30 μL of pretreated sample of blood was taken in glass vial and extracted in to a 2 μL of organic solvent containing the Cyanex-302 as an extracting agent at the optimized condition of the method, and then analyzed with GF-AAS. The pretreated blood samples were diluted with distilled water in order to bring the concentration in the range of calibration curve. The concentration of lead was calculated by using calibration curve which was drawn in sample solution. The level of lead found in different blood samples is given in Table 2. To verify the validity of the proposed method, known amounts of lead were added into the blood sample in order to evaluate the recovery percentage (%). For, this 10 and 25 ng mL^{-1} of lead were spiked in to the blood sample and the actual concentration of lead was determined by using present method while considering the dilution factor of the sample. The recovery of lead in blood was found to be 91.6–97.0% with precision of 3.5–6.2%. The value of recovery % found in blood is given in Table 3.Further, the accuracy of the method was also confirmed by the determination of lead in CRM of bovine blood samples by using the calibration curve while considering the dilution factor of the sample. The results are given in Table 4. The concentra-

Table 2
Determination of lead in blood samples, ng mL^{-1}.

Samples source/Age	Dilution factor	Lead	RSD (%)
Male/35	2	50.5	3.8
Male/28	2	35.5	4.5
Male/14	2	30.1	3.7
Female/45	2	46.2	4.7
Female/32	–	15.5	3.0

Table 3
Determination of recovery % of lead in blood samples.

Sample source/Age	Lead found by present method ($ng\,mL^{-1}$) (a)	Lead added ($ng\,mL^{-1}$) (b)	Dilution factor	Total lead found ($ng\,mL^{-1}$) (c)	Recovery (%) $(c-a)/b \times 100$
Male/20	30.4	25.0	2	53.8	93.6
	30.2	10.0	2	39.9	97.0
Female/26	42.5	25.0	4	65.4	91.6
	42.5	10.0	2	51.8	93.0

Table 4
Determination of lead in CRM bovine blood samples.

Sample	Dilution factor	Lead by proposed method ($ng\,mL^{-1}$)	Certified value of lead ($ng\,mL^{-1}$)
Lot No. 194	5	121 ± 3	126 ± 4
Lot No. 195	15	411 ± 7	416 ± 9

Table 5
Comparison of proposed methods with other reported methods.

Techniques	PF	LOD ($ng\,mL^{-1}$)	Volume of sample (μL)
SDME [20]	52	0.2	2000
DLLME [21]	78	0.04	5000
CPE [22]	50	0.08	10,000
DDSME [proposed method]	13	0.08	30

tion of lead found in CRM materials was very close to the certified values. Thus, the use of DDSME method could acts as an excellent sample cleanup method to eliminate various matrices present in samples as well as a concentrating probe to determine the trace level of lead in blood.

3.7. Comparison of proposed method with other reported methods

The potentiality of the present method was compared with the results of earlier liquid-phase extraction methods for the determination of lead [20–22]. Table 5 displays the comparison of LOD, preconcentration factor and volume of sample used for extraction of lead. The value of preconcentration factor obtained by present method was less than other reported methods probably due to the use of micro amount (30 μL) volume of sample for extraction of lead. The value of LOD obtained by the present method was comparable to reported methods. The present method used micro amount of sample solution for the separation and preconcentration of lead from biological samples as compared to other liquid-phase microextractions.

4. Conclusions

The proposed method has been successfully applied for the determination of lead by using micro liter volume of human blood samples. Applying this method prior to GF-AAS helps in the preconcentration of analytes and also provides an excellent sample cleanup procedure by excluding various complex matrices present in the blood samples. The advantages of present method are simple, rapid, low cost and little solvent and sample consumption for monitoring of lead in biological samples. In future, this technique could be applied for pharmacokinetic and clinical studies are expected.

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